Effects of Dietary Yeast Supplementation on Serum Immunoglobulin Concentrations in Quarter Horse Mares

Thesis

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By

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ABSTRACT

Dietary yeast supplementation has been reported to influence immune responses in multiples species, including horses, with mixed results. In this study, sixteen Quarter Horse mares (10.6 ± 5.0 yrs.) were used to evaluate the effect of dietary yeast supplementation on immunoglobulin concentrations in response to vaccination. Mares were blocked by reproductive status and diet and randomly assigned to one of two treatment groups: Yeast or Control. Open mares received 0.5% BW of a 12% CP pelleted concentrate while pregnant mares received 0.5% BW of a 16% CP pelleted concentrate. All horses also received mixed grass hay and water *ad libitum.* Horses in the yeast treatment group were fed a target dose of 1 g/45.4 kg of BW per day of a live culture of *Saccharomyces cerevisiae* throughout the study. After 60d (d 300 of gestation), mares were vaccinated with a commercial equine tetanus vaccine and blood samples were taken via jugular venipuncture immediately prior to vaccination (d 0) and on d 7, 14, 21 and 28 post-vaccination. Sera samples were measured for IgG(T), IgGa, IgGb, IgA, and IgM antibodies using an ELISA assay and data were analyzed using the PROC MIXED in SAS. A P value of ≤ 0.05 was considered statistically significant. Prior to vaccination, open mares tended to have higher IgG(T) specific antibody titers compared to pregnant mares (P = 0.07). Previous research has shown that IgG antibody titers increase in response to vaccination with a variety of antigens. However, in this study, IgG(T) specific antibody titers decreased in response to vaccination, regardless of reproductive status or yeast supplementation. There was a difference due to diet (P = 0.002) but not when pregnancy was also included (P = 0.0775) There were no differences due to diet regardless of pregnancy with regard to IgA, IgM, IgGa, and IgGb. Overall, the dose of 1 g/45.4 kg of BW per day of dietary yeast influence IgG(T) but not IgA, IgM, IgGa, and IgGb specific antibody response in this study.

Key words

Immunoglobulins, IgG(T), IgGa, IgGb, IgA, IgM, antibodies, horse, ELISA, yeast, *Saccharomyces cerevisiae*

INTRODUCTION

In the early 1970's, many of the equine immunoglobulins were identified leading to the classification of IgGa, IgGb, IgG(T), IgA, and IgM (McGuire et al., 1973). Immunoglobulin (Ig) G is a major immunoglobulin found in equine serum (Sheoran et al, 1998). In general, IgG provides the longest protection of the immunoglobulins and is usually the first circulating antibody during a secondary immune response, or second exposure to a specific antigen (Nester, 2004). Vaccination and immunization take advantage of the secondary response and can be used as a way to elicit an immune response (Nester, 2004). IgG protects the body through mechanisms such as complement activation, opsonization, aggregation, and pathogen immobilization. Recently, the subisotypes of equine IgG have been further divided into seven different isotypes due to the seven different genes that code for the constant heavy chain regions (Wagner et al, 2004). Predominant IgG subisotypes in horse serum are IgGa, IgGb, and IgG(T) with IgGb being the most prominent followed by IgG(T) and lastly, IgGa (Sheoran et al 2000; Lewis et al., 2008). IgG(T) refers to the immunoglobulin that comes from the exposure to tetanus toxoid immunization (Weir et al., 1966; Widders et al., 1986). IgGa and IgGb were differentially characterized by specific binding characteristics to staphylococci proteins A and G (Sheoran et al, 1996). IgGa is represented by one subclass, while both IgGb and IgG(T) are each represented by two of these subclasses (Wagner et al, 2006). However, since the classification of the different IgG subclasses, research involving mechanisms and specific functions of these subclasses is still largely unknown.

Immunoglobulin A (IgA) is associated with mucosal immunity and is known as the secretory immunoglobulin. In the horse, IgA can be found in the milk as well as other secretory areas such as the nasal passages (Lewis et al, 2010). Declines in serum IgA may be correlated with increased immunodeficiency symptoms (Flaminio et al., 2009). IgA levels have also been indicated as a means of evaluating the equine immunity (Tallmadge et al., 2009). Immunoglobulin M is a pentameric immunoglobulin and is often the first immunoglobulin produced in response to antigens. Due to the large structural size of IgM, this class of immunoglobulin acts primarily with infections in the blood stream and often elicits the classical pathway of the complement system (Nester, 2004).

Equine immunoglobulins do not cross the placenta. Immunoglobulins are passively transferred to the foal through the mother's milk, or colostrum. This passive transfer of immunity to young foals causes industry interest in increasing serum IgG concentrations in pregnant mares (McGuire et al., 1973; Bondo et al., 2011). Foals need to ingest enough colostrum containing immunoglobulins to prevent failure of passive transfer of antibodies to protect them from potential pathogenic invasion early in the life (McGuire et al., 1977; Crawford and Perryman, 1980). Failure of passive transfer of immunoglobulins from mare to foal can be a significant and common immunodeficiency problem in horses that causes industry concern.

In previous research studies, dietary yeast supplementation has been shown to act as an immunostimulant when fed daily in dairy cows. A slight increase in IgG subisotypes was seen with a slight decrease in non-specific IgA (Cakiroglu et al., 2010). Studies have shown that *Saccharomyces cerevisae* may elicit inflammatory immune responses and reduce mortality due to immune response in species other than horses such as pigs and feedlot steers (Emmanuel et al.,

2007; Collier et al., 2011). Components of the yeast cell wall such as mannons and beta-glucans may be responsible for these immunostimulatory results (Kogan et al., 2007;Wismar et al., 2010). However, little published research has been conducted with regard to horses.

Horses are largely used for both recreational and breeding purposes. These two uses for horses, specifically mares, led to an interest in immunoglobulin levels for both pregnant and open (non-pregnant) mares. In the equine industry, dietary supplementation with commercially available products has become an increasingly popular way to attempt to improve animal health. The current study intended to examine the effects of commercially available yeast supplementation on the immune response associated with immune parameters such as immunoglobulins and more specifically IgG(T), IgGa, IgGb, IgA, and IgM in response to vaccination.

MATERIALS AND METHODS

Horses and Supplementation – Sixteen Quarter Horse mares (10.6 ± 5.0 yrs.) were used to evaluate the effect of dietary yeast supplementation on IgG(T), IgGa, IgGb, IgA, and IgM specific antibody responses. Mares were blocked by reproductive status and diet and randomly assigned to one of two treatment groups: Yeast or Control. Open mares will receive 0.5% BW of a 12% CP pelleted concentrate while pregnant mares will receive 0.5% BW of a 16% CP pelleted concentrate. All horses received mixed grass hay and water *ad libitum.* Horses in the yeast treatment group were fed a target dose of 1 g/45.4 kg of BW per day of a live culture of *Saccharomyces cerevisiae* throughout the study.

Vaccination and Serum Collection - After 60d (d 300 of gestation) of supplementation, mares were vaccinated with a commercial equine tetanus vaccine and blood samples were taken via jugular venipuncture immediately prior to vaccination (d 0) and on d 7, 14, 21 and 28 post-vaccination. Blood samples were centrifuged at 10,000 rpm for 5 minutes and serum was decanted. Serum samples were then stored at -80^{o}C until further analysis.

ELISA Kits –Serum samples were evaluated by the use of commercially available kits for equine serum: Horse IgG(T) ELISA Quantitation Set; Bethyl Laboratories Cat. No. E70-105, Horse IgA ELISA Quantitation Set; Bethyl Laboratories Cat. No. E70-116, Horse IgM ELISA Quantitation Set; Bethyl Laboratories Cat. No. E70-114, Horse IgGa ELISA Quantitation Set; Bethyl Laboratories Cat. No. E70-124, Horse IgGb ELISA Quantitation Set; Bethyl Laboratories Cat. No. E70-127, and four parameter logistics curves. Serum samples were diluted in order to fit the curve set by the standards. Samples were run in duplicate for each ELISA. All duplicate values were within 5% of each other.

Data Analysis – Data were analyzed using the MIXED procedure of SAS v 9.3 (SAS Institute Inc., Cary, N.C.). The model included the fixed effects of diets (1 df), pregnancy status (1 df), days of sampling (4 df) and all 2-way and 3-way interactions, plus a covariate measurement taken at the initiation of the trial (1 df), and the random effects of mare nested within diet and pregnancy(3 df) and the residual error (28 df). The covariance structures were modeled using the CSH with IgG(T), CS with IgA, and AR(1) with IgGa, IgGb, and IgM structure of errors. The decision in regards to the best error structure was made based on the smallest Bayesian Information Criterion. Comparisons

of the two diet treatments across all sampling days were made by decomposing the diet x days term into single degrees of freedom contrasts (i.e., using the SLICE option of the LSMEANS statement). Other mean comparisons were made using Fisher's protected least-significant difference (LSD). Significance was declared at $P < 0.05$. All results are expressed as least-squares means with the respective standard errors of the least means squares.

RESULTS

Serum IgG(T) Concentration

Serum IgG(T) concentration between Yeast and Control mares at each time point are shown in **Figures 1A and 1B**. Open mares are presented in **Figure 1A.** Similar IgG(T) concentrations were seen d0 for both groups. Concentrations continued to be similar throughout the duration of the study; however, the trend for the two groups differed. The control diet group of mares exhibited a steady decline in serum IgG(T) concentration at all time points post-vaccination ($P < .0001$). A slight increase at d28 post-vaccination was seen for the open yeast supplemented mares.

Pregnant mares are presented in **Figure 1B**. Pregnant mares that received the control diet appeared to start with a much lower IgG(T) concentration than the yeast supplemented group of mares. Concentrations between groups became more similar post d14. Again, the trends between the two groups differed. The pregnant mares that received the yeast diet exhibited a decrease until d21 and then a slight increase d28 post-vaccination. The pregnant mares that received the control diet exhibited a trend that increased serum IgG(T) concentration until d14 and then a steady decrease was observed

through d28. Peak IgG(T) production appeared to be at d14 for the pregnant mares that received the control diet; however, peak production for the mares that received the yeast diet appeared to be day of vaccination.

Among the different groups, the two yeast supplemented groups showed similar trends with slightly less variation between the pregnancy statuses of the animals. The control groups showed entirely different trends. **Figure 6A** shows IgG(T) concentrations for all four treatment groups at all time points. Overall, differences between diet were not significant when pregnancy status was taken into account (P = 0.0775).

Serum IgGa Concentrations

Serum IgGa concentration between Yeast and Control mares at each time point are shown in **Figures 2A and 2B**. The only difference was with regard to day post vaccination (P = 0.0148).There did not appear to be any differences due to dietary supplementation with regard to IgGa concentration (P = 0.481) Open mares are presented in **Figure 2A**. There was no observable trend among the open mares that received the control diet. The open mares that received the diet containing yeast showed a slight trend. Serum IgGa concentration appeared to increase from d0 to d14, then decrease d14 to d28. Both Yeast and Control groups appeared to peak d14 post-vaccination.

Pregnant mares are presented in **Figure 2B**. Pregnant mares that received the control diet showed an observable trend with an increase D0 to D14 and then a decrease d14 to d21 with a slight increase at d28. There was no observable pattern for the pregnant mares that received the yeast supplement. dO and d14 had similar IgGa concentrations. Both pregnant groups also peaked at d14.

Between the groups, trends were also similar. The open mares that received yeast exhibited a similar trend to the pregnant mares that received the control diet from d0 to d21. Similar trends were also seen d0 to d21 between the open control group and the pregnant yeast group. All groups saw a peak in serum IgGa concentration at d14. **Figure 6B** shows IgGa concentrations for all four groups at all time points.

Serum IgGb Concentrations

Serum IgGb concentration between Yeast and Control mares at each time point are shown in **Figures 3A and 3B**. No differences were seen between treatments (P = 0.1326) or day post-vaccination (P = 0.1195) with regard to IgGb concentration. Open mares are presented in **Figure 3A**. Open mares that received the control diet showed a slight trend with an increase d0 to d14; however, the peak IgGb concentration was not seen until d28. The open mares that received the yeast supplement peaked at d14 then proceeded to decrease to d28. Both open groups had similar IgGb concentration on d7 and d21.

Pregnant mares are presented in **Figure 3B**. Pregnant mares that received the control diet peaked at d14, then proceeded to decrease to decrease to d28. The pregnant mares that received yeast did not show an observable pattern.

Between the four different groups, more variation was seen across the pregnant groups than was seen across the open groups. Trends were similar between the open yeast and pregnant control groups d14 to d28; both of which peaked at D14. **Figure 6C** shows the IgGb concentration for all four groups at all time points.

Serum IgA Concentrations

Serum IgA concentration between Yeast and Control mares at each time point are shown in **Figures 4A and 4B**. No differences were seen between treatments (P = 0.7901) or day post-vaccination (P = 0.6573) with regard to IgA concentration. Open mares are presented in **Figure 4A**. Open mares that received the control diet did not show an observable pattern; however, concentrations on d7 and d21were similar. The open mares that received the yeast supplement showed a relatively steady increase in IgA concentration from d0 through d28. Both open groups showed little variation d0, d14, and d28.

Pregnant mares are presented in **Figure 4B**. Pregnant mares that received the control diet showed a relatively steady increase d0 through d28. Pregnant mares that received the yeast supplement had similar IgA concentrations on d14 and d21.

Among the four groups, more variation was seen in pregnant rather than open groups. The trends in IgA concentration for open yeast mares and the pregnant control mares were similar. **Figure 6D** shows the IgA concentrations for all four groups.

Serum IgM Concentrations

Serum IgM concentration between Yeast and Control mares at each time point are shown in **Figures 5A and 5B**. No differences were seen between treatments (P = 0.7752) or day post-vaccination (P = 0.1451) with regard to IgM concentration. Open mares are presented in **Figure 5A**. Open mares that received the control diet showed a peak of IgM at d7 with a decrease from d7 to d28. The open mares that received the yeast supplement showed a relatively steady increase d0 through d28.

Pregnant mares are presented in **Figure 5B**. Pregnant mares that received the control diet had peak IgM levels at d14, with a steady increase to that time point. The group that received yeast had a lesser increase through d14; however, both pregnant groups showed a peak at d14. Between all four groups, limited similarities were apparent. **Figure 6E** shows the IgM concentrations for all four groups.

DISCUSSION

Nutritional immunology or the use of nutritional products used to enhance the immune response has become an increasingly more popular means of enhancing previously used vaccines. Supplementation with a variety of products such as yeast and vitamin E has gained popularity among livestock owners. A variety of companies have been developing such supplemented products as well as potential doses. For the current study, a target dose of 1 g/ 45.4 kg BW was consistent with the manufacturer's instruction of a target dose of 10 g/ 454 kg BW or close to 10 g/ day. Previous research in dairy cattle suggested that this dose was able to increase IgG serum concentration (Cakiroglu et al., 2010); however, in the current study, no differences between serum concentration and yeast supplementation were observed. Potentially, a higher dose of yeast may be necessary to elicit such an immune response.

Overall, the levels of immunoglobulins seen in this study were consistent with previous studies with ranges between 200 mg/dL and 2000 mg/ dL for horses age 5-12 years (McFarlane et al., 2001; Mizukoshi et al, 2002; Tizzard, 2004; Petersson et al, 2010). IgGa, IgM, and IgG(T) tended to have slightly higher levels than previously seen with

immunostimulant supplementation (Petersson et al, 2010). This may have occurred because low serum levels of yeast may elicit an immune response through opsonization (Grondahl et al., 2001). IgGb tended to have lower serum levels than previously seen with the feeding of an immunostimulant (Petersson et al, 2010); however, this may have occurred because IgGb may be correlated with parasitemia control (Cunha et al., 2006).This may also partially explain why IgG(T) levels were high prior to vaccination. IgG(T) did not appear to increase throughout this study until near d28 post-vaccination. There appeared to be a steady decrease in IgG(T) concentration until d28 for the two yeast supplemented groups. Previous research has shown that IgG(T) may take over 4 weeks to peak post-infection or may not change in titer level over many months post-immunization (Cunha et al., 2006). The Pregnant control group did exhibit a peak in IgG(T) production near d14 post-vaccination. The remaining three treatment groups had the highest level d0 post-vaccination. The two yeast supplemented groups showed similar trends for IgG(T) with limited variation. Yeast supplementation may delay the peak production of IgG(T) post-vaccination until d28.

Peak production of IgGa and IgGb seemed to occur near d14 for most of the treatment groups with the exception of open control group for IgGb. This is consistent with previous research involving immune responses to equine herpes virus as well as vaccination (Mizukoshi et al., 2002; Sheoran et al., 2003). Drawing comparisons between acute infection and vaccination is viable (Cunha et al., 2006). IgM peaked near d14 post-vaccination in both pregnant groups which is consistent with supplementation and vaccination response in older mares (Petersson et al., 2010). In the open control group, the peak was near d7, which is consistent with the well-known convention that IgM is the first antibody produced (Nester, 2004). Potentially pregnancy and yeast supplementation may delay the production of IgM.

In the serum, overall levels of IgG(T), IgGa, and IgGb appeared similar. Previous research indicated that IgGb is the predominant subisotype of IgG in equine serum; however, in this study, a large difference in the subisotypes was not seen (Petersson et al, 2010; Lewis et al., 2008; Sheoran et al 2000). Other studies have shown that when challenged with immunological stimulants other than vaccination, IgG(T) may be the predominate subisotype as well as fluctuate in a manner that was seen in this study (Dowdall et al.,2002).

Since IgA is the predominant immunoglobulin in nasal secretions rather than serum, different patterns in response to vaccination may have been seen if nasal swabs were analyzed along with serum (Sheoran et al., 2003). This may have given a more applicable analysis of IgA in response to yeast supplementation and vaccination; however, there were detectable levels of IgA in the serum and the concentration did appear to increase and slightly fluctuate.

Overall, trends were similar for open yeast and pregnant control groups for IgGa, IgA, and IgGb. This may indicate that yeast supplementation in the open mares may mock the immunological effects of pregnancy. IgG(T) appeared to show different and almost opposite trends than what was seen for IgGa and IgGb. This may be partially due to the fact that IgG(T) levels are inversely related to IgG levels in equine serum (McGuire et al., 1971). This opposite or different trend may be due to the functionality of the separate subisotypes of equine IgG. IgG(T) may inhibit the action of IgGa and IgGb by competitively binding antigen (McGuire et al., 1971; Lunn et al., 1998). IgM did not appear to show many trends across treatment groups.

CONCLUSION

Overall, dietary yeast supplementation did not influence serum IgGa, IgGb, IgA, or IgM but did influence serum IgG(T) concentration in response to vaccination when fed at a target dose of 1 g/45.4 kg BW once daily.

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FIGURES

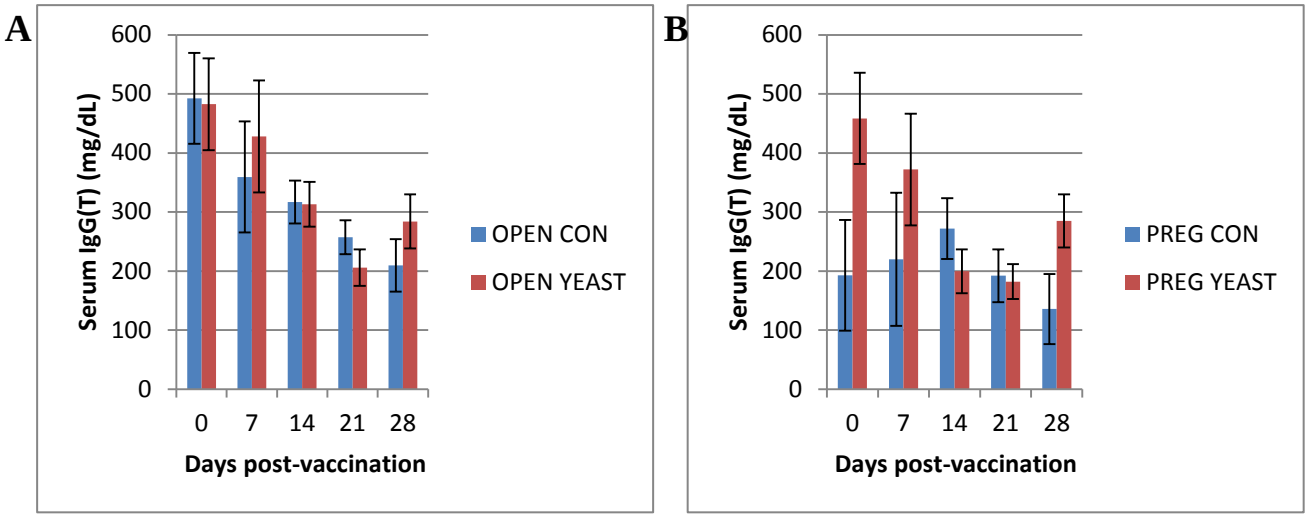

Figure 1.

Comparison of serum IgG(T) concentration in mg/dL of open **(A)** and pregnant **(B)** mares between control and yeast supplemented diets from day of vaccination through D28 post vaccination (n= 4 per time point per group). Bars represent mean ± SE. Day Post Vaccination (PV) P = <.0001; DIET*DayPV P = 0.002; PREG*DayPV P = 0.3328; DIET*PREG*DayPV P = 0.0775.

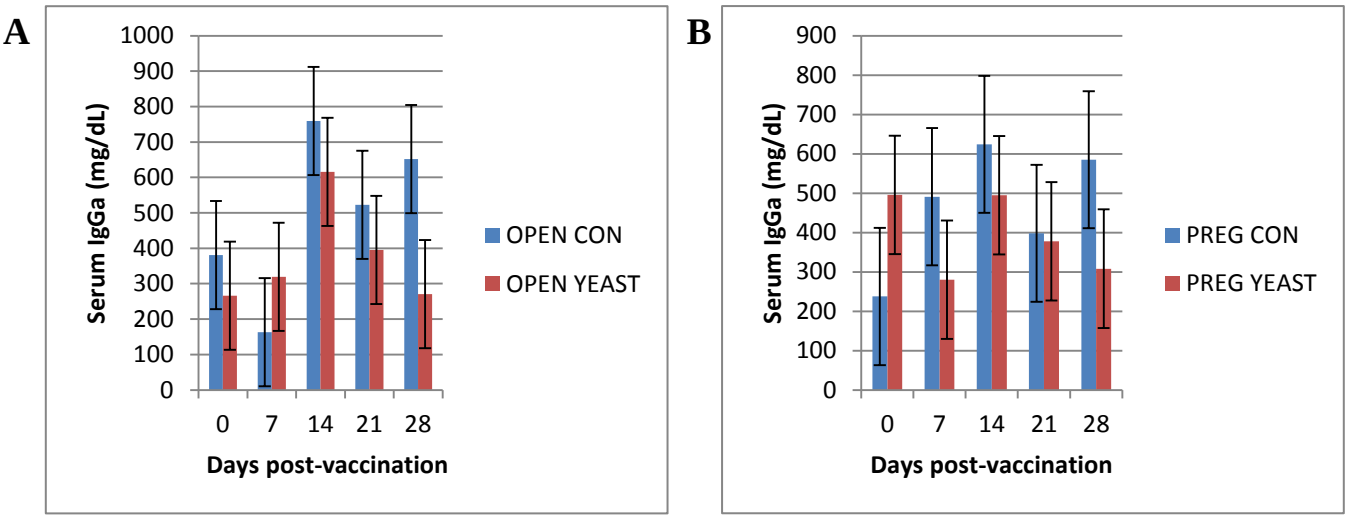

Figure 2.

Comparison of serum IgGa concentration in mg/dL of open **(A)** and pregnant **(B)** mares between control and yeast supplemented diets from day of vaccination through D28 post vaccination (n= 4 per time point per group). Bars represent mean ± SE. Day Post Vaccination (PV) P = 0.0148; DIET*DayPV P = 0.2845; PREG*DayPV P = 0.7247; DIET*PREG*DayPV P = 0.4805.

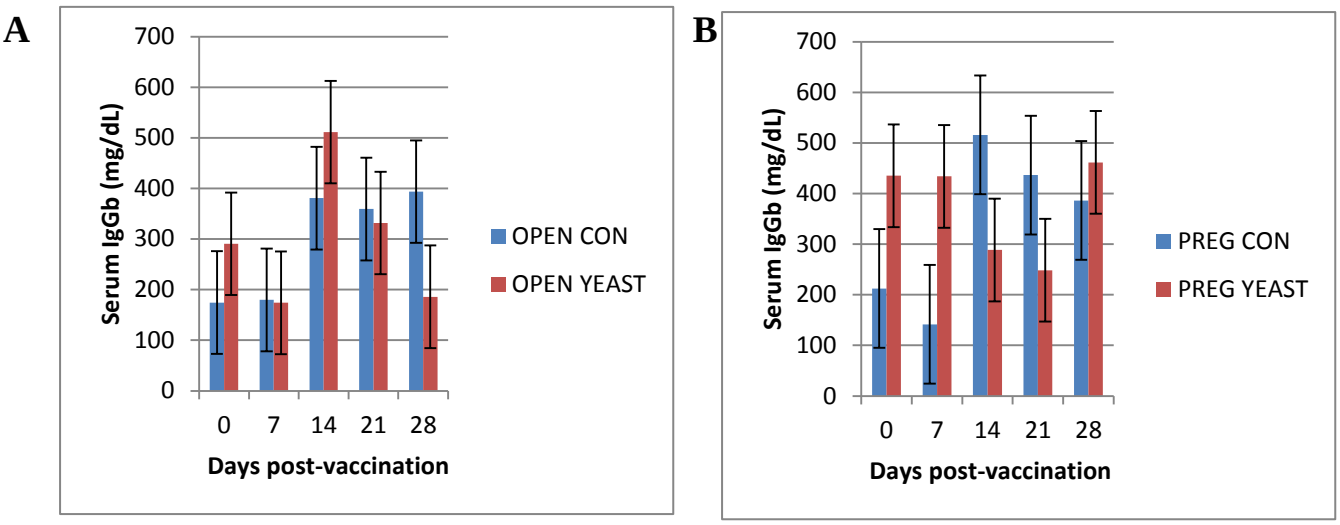

Figure 3.

Comparison of serum IgGb concentration in mg/dL of open **(A)** and pregnant **(B)** mares between control and yeast supplemented diets from day of vaccination through D28 post vaccination (n= 4 per time point per group). Bars represent mean ± SE. Day Post Vaccination (PV) P = 0.1195; DIET*DayPV P = 0.2238; PREG*DayPV P = 0.708; DIET*PREG*DayPV P = 0.1326.

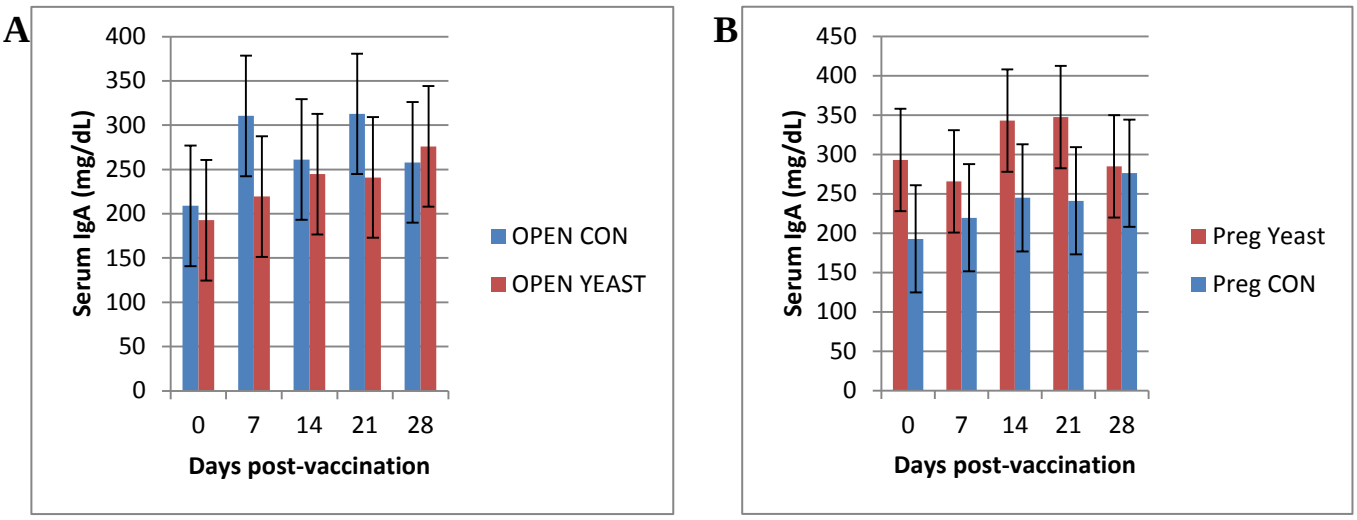

Figure 4.

Comparison of serum IgA concentration in mg/dL of open **(A)** and pregnant **(B)** mares between control and yeast supplemented diets from day of vaccination through D28 post vaccination (n= 4 per time point per group). Bars represent mean ± SE. Day Post Vaccination (PV) P = 0.6573; DIET*DayPV P = 0.6728; PREG*DayPV P = 0.8094; DIET*PREG*DayPV P = 0.7901.

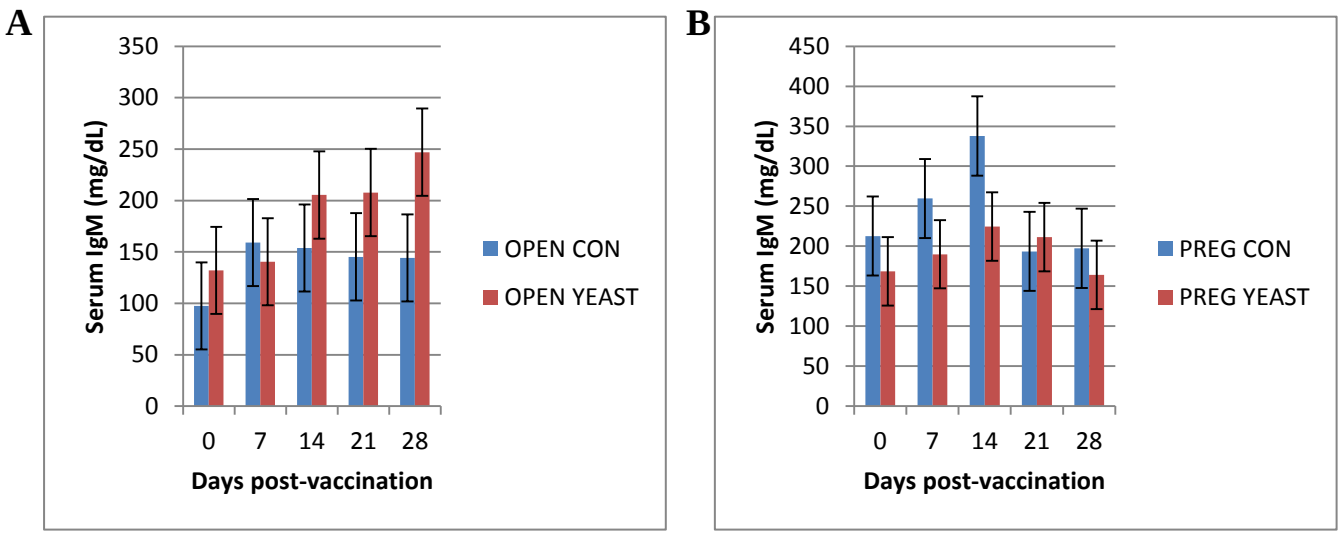

Figure 5.

Comparison of serum IgM concentration in mg/dL of open **(A)** and pregnant **(B)** mares between control and yeast supplemented diets from day of vaccination through D28 post vaccination (n= 4 per time point per group). Bars represent mean ± SE. Day Post Vaccination (PV) P = 0.1451; DIET*DayPV P = 0.5319; PREG*DayPV P = 0.3462; DIET*PREG*DayPV P = 0.7752.

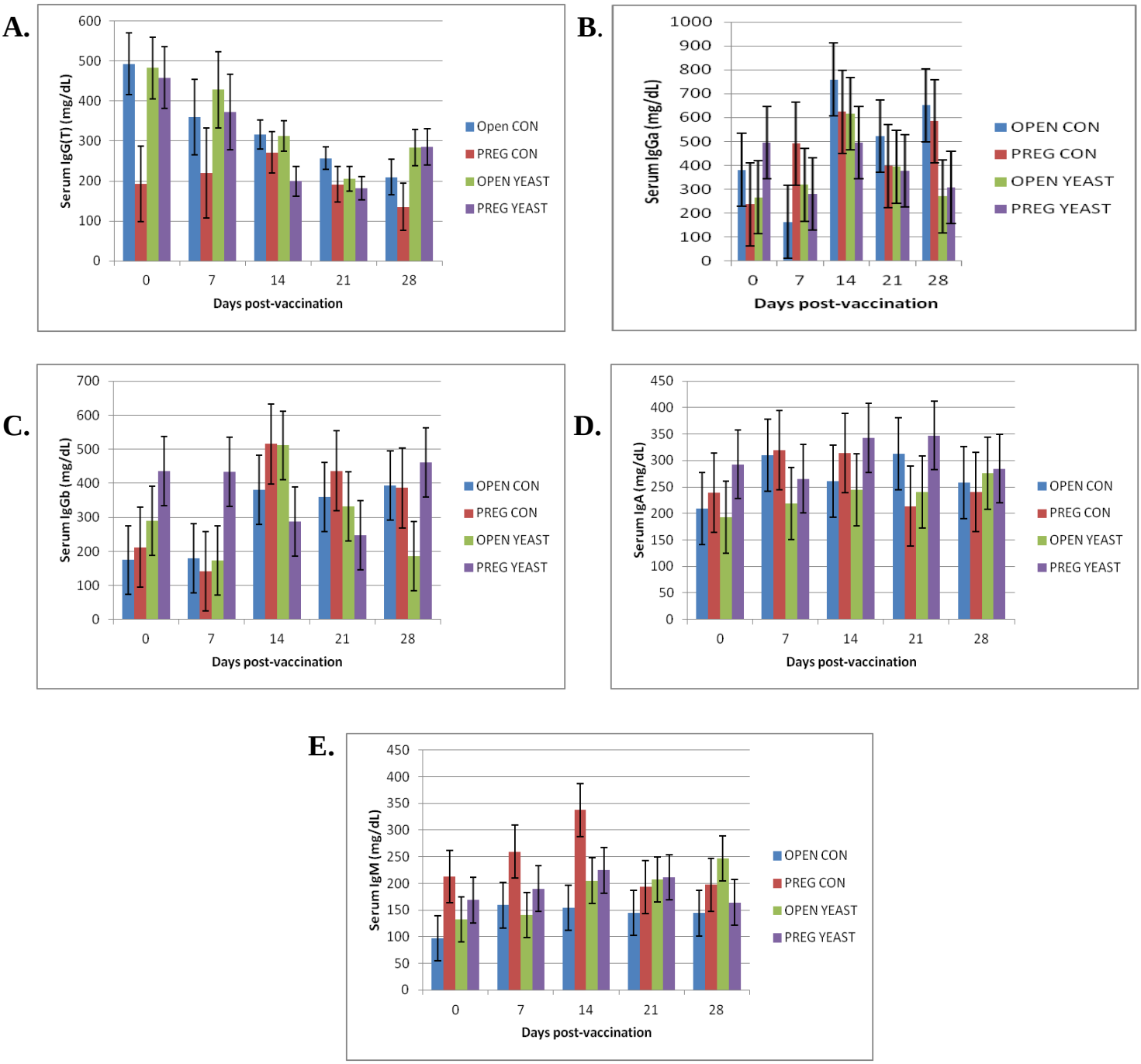

Figure 6.

Comparison of serum immunoglobulin concentration including IgG(T) **(A)**, IgGa **(B)**, IgGb **(C)**, IgA **(D)** and IgM **(E)** of four treatment groups from day of vaccination through D28 post

vaccination (n= 4 per time point per group). Bars represent mean ± SE. P values shown in **Figures 1-5.**